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Effect of heparin on the glomerular structure and function of remnant nephrons

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Effect of heparin on the glomerular structure and function of remnant nephrons. The development of glomerular structural abnormalities in remnant nephrons, after ablation of renal mass (subtotal nephrectomy), in rats is largely prevented by the daily injection of heparin. To investigate if this protective effect of heparin is due to attenuation of glomerular hyperperfusion, hypertension and hyperfiltration, which develop in remnant nephrons soon after subtotal nephrectomy, we measured various parameters of glomerular hemodynamics at two weeks (Group 1) and four weeks (Group 2) after removal of 1-3/4 of total kidney mass in heparin-treated (Groups 1A and 2A) and untreated (Groups 1B and 2B) Munich-Wistar rats. When compared to normal non-nephrectomized rats (Group 1C), the values for glomerular capillary hydraulic pressure (P_{GC}), glomerular plasma flow rate (Q_A) and single nephron filtration rate (SNGFR) in remnant nephrons were found to be markedly and similarly elevated in both Groups 1A and 1B, averaging 71 ± 4 and 73 ± 4 mm Hg, 229 ± 41 and 176 ± 13 nl/min, 58.9 ± 6.4 and 60.8 ± 7.8 nl/min, respectively. Thus, glomerular hemodynamic parameters two weeks after subtotal nephrectomy did not differ between untreated and heparin-treated rats. Likewise, heparin treatment did not decrease the values of P_{GC} and SNGFR assessed four weeks after subtotal nephrectomy, with the average values being 65 ± 2 mm Hg and 83.8 ± 7.1 nl/min in Group 2A versus 62 ± 4 mm Hg and 63.7 ± 6.5 nl/min in Group 2B. Thin-section light microscopic studies revealed that most of the remnant glomeruli of heparin-treated rats remained structurally intact throughout the entire study period of 7.5 weeks. In non-treated rats progressive destruction of glomerular architecture developed and normal glomeruli comprised 23 ± 7% of the total remaining nephron population at 7.5 weeks after subtotal nephrectomy. Therefore, the effect of heparin in protecting glomeruli of remnant nephrons from structural damage is mediated by a mechanism independent of glomerular hyperperfusion, hypertension or hyperfiltration occurring in these nephrons. The importance of an anticoagulant and/or antiproliferative effect of heparin is suggested as playing a role in the protection of glomerular structure.

Subtotal renal ablation, as a result of unilateral nephrectomy and segmental infarction of the contralateral kidney, leads to several functional and structural features characteristic of chronic renal failure [1–11]. These include the development of azotemia, which is progressive in nature, proteinuria and, histologically, hyalinization and obsolescence of glomeruli. Moreover, nephron function prior to the development of structural damage is characterized by elevated levels of both filtration and glomerular capillary hydraulic pressure (P_{GC}), features reported in other experimental models of chronic renal disease [12, 13]. For these reasons, the model of subtotal renal ablation has been extensively used in recent years to explore the mechanisms underlying the progressive nature of chronic renal failure. More recently, micropuncture studies have demonstrated a close correlation between the level of P_{GC} measured in the early phase of subtotal renal ablation and the degree of glomerular structural damage found later [3, 5, 8, 9]. A few maneuvers which dampen the increase in P_{GC} were also shown to substantially limit the subsequent glomerular structural alterations [3, 5, 8, 9]. Treatment with heparin [2, 6, 14], and the administration of OKY1581 [7], an inhibitor of thromboxane synthesis, have been shown to have a protective effect on renal histology after subtotal renal ablation in rats. Since these two agents share a common functional feature, that is, interfering with the blood coagulation cascade, it has been suggested that local activation of the coagulation process may be involved in the structural alterations of glomeruli seen in this model.

Heparin administration in previous studies [2, 6] also markedly attenuated the development of systemic hypertension in rats with subtotal nephrectomy. Thus, the effect of heparin in ameliorating the development of glomerulosclerosis may be due to its ability to prevent the hemodynamic abnormalities (mainly intraglomerular hypertension) which develop in the remnant nephrons of rats with subtotal nephrectomy. Alternatively, heparin may prevent glomerulosclerosis by effects acting distal to the develpment of hemodynamic abnormalities. The present studies were designed to examine these possibilities.

Methods

Study groups

All experiments were performed in adult Munich-Wistar rats weighing 118 to 223 grams. All animals were allowed free access to regular rat chow and tap water throughout the experiments, and all but Group 1C animals underwent subtotal nephrectomy. Table 1 summarizes the specific protocol employed in the seven study groups. Rats of Groups 1A, 2A and 3A were given 100 U of heparin sodium (derived from beef lung) (VHA + Plus™, Lot Number V38610, Lyphomed, Inc., Melrose Park, Illinois, USA) subcutaneously twice daily, beginning 24 hours after

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Table 1. Summary for the protocols for experimental groups

Group	N =	Procedure	Time of experiment	Parameters measured
1A	6 rats	Subtotal NPX + heparin	After 2 wks of treatment	Glomerular hemodynamics (eg. SNGFR, P_{GC},Q_A,K_f,R_A,R_E)
1B	6 rats	Subtotal NPX + vehicle	2 wks after subtotal NPX	Glomerular hemodynamics (eg. SNGFR, P_{GC},Q_A,K_f,R_A,R_E)
1C	7 rats	Normal controls[a]	Age-matched with Group 1A & 1B	Glomerular hemodynamics (eg. SNGFR, P_{GC},Q_A,K_f,R_A,R_E)
2A	10 rats	Subtotal NPX + heparin	After 4 wks of treatment	SNGFR, P_{GC}, P_T
2B	8 rats	Subtotal NPX + vehicle	4 wks after subtotal NPX	SNGFR, P_{GC}, P_T
3A	6 rats	Subtotal NPX + heparin	After 7.5 wks of treatment	Histology
3B	6 rats	Subtotal NPX + vehicle	7.5 wks after subtotal NPX	Histology

Abbreviations are: NPX, nephrectomy; SNGFR, single nephron filtration rate; P_{GC}, glomerular capillary hydraulic pressure; P_T, proximal tubule or Bowman's space hydraulic pressure; Q_A, glomerular plasma flow rate; K_f, glomerular capillary ultrafiltration coefficient; R_A, afferent arteriolar resistance; R_E, efferent arteriolar resistance.

[a] This group of rats was not studied contemporaneously with the other groups. It was added at the request of the referees during the review process of this paper.

subtotal nephrectomy and continued until 12 hours prior to micropuncture and/or histologic studies. Micropuncture measurements or histological examination were performed 2, 4 and 7.5 weeks after subtotal nephrectomy in rats of Groups 1, 2 and 3, respectively. Rats in Group 1C, which were age-matched with Groups 1A and 1B, did not have subtotal nephrectomy and served as controls. As outlined also in Table 1, micropuncture measurements were performed only in Groups 1 and 2. In Group 2, micropuncture assessments were made only for SNGFR, P_{GC} and P_T.[1] The remnant kidneys of Groups 3A and 3B animals were subjected to histological examination at the completion of the study, using the protocol described below.

Experimental procedures

Subtotal nephrectomy. All animals underwent subtotal nephrectomy. For this purpose, rats were anesthetized with ether. All surgical tools were sterilized with 70% ethyl alcohol. Subtotal nephrectomy was performed by right nephrectomy and the ligation of at least three branches of the left renal artery with 4-0 silk to allow approximately 1/4 of single kidney tissue mass to remain viable. Care was taken to preserve the adrenal glands during surgery. After the procedures described above, the abdominal incision was closed with 4-0 silk (Ethicon, Inc., Somerville, New Jersey, USA), and stainless steel wound clips were used to close the skin. After animals recovered from the anesthesia, they were returned to the cages.

Preparatory surgery for micropuncture. On the day of the experiment, rats were anesthetized with Inactin (100 mg/kg body wt, i.p.) and surgically prepared in a fashion routinely performed in our laboratory [15]. Briefly, following tracheostomy, indwelling polyethylene catheters (PE-50, Clay Adams, Parsippany, New Jersey, USA) were placed into the left and right jugular and left femoral veins for subsequent infusion of plasma, whole blood, inulin and/or dextran solution. The left femoral artery was also catheterized to monitor mean arterial pressure (MAP) and for periodic blood collections. MAP was measured by an electronic transducer (Model p23Db; Gould Inc., Cleveland, Ohio, USA) connected to a recorder (Model 2200S; Gould Inc.). A laparotomy was then performed. The left ureter was catheterized with a polyethylene tube (PE-10) for subsequent urine collections and the left kidney was immobilized for micropuncture study. To maintain the circulating plasma volume at a normal euvolemic level during the experiment, each rat received iso-oncotic rat plasma in a volume of 10 ml/kg intravenously over the initial 30 minutes, followed by continuous infusion at the rate of 0.6 ml/kg/hr [15]. For the estimation of GFR and single nephron glomerular filtration rate (SNGFR), 9% inulin in a 0.9% NaCl solution was infused intravenously with a priming dose of 0.4 ml, followed by constant infusion at a rate of 1.2 ml/hr.

Following the above preparatory procedures, rats were subjected to the micropuncture study specified below.

Micropuncture measurement of vairous glomerular microcirculatory parameters. Using the method previously described in detail [15, 16], micropuncture measurements and collections were made in all rats of Groups 1A, 1B and 1C to determine the following: SNGFR, P_{GC}, proximal tubule (P_T) and surface efferent arteriolar hydraulic pressure (P_E), femoral arterial (C_A) and efferent arteriolar (C_E) plasma protein concentrations, single nephron filtration fraction (SNFF), initial glomerular capillary plasma flow rate (Q_A), glomerular capillary ultrafiltration coefficient (K_f), as well as resistances of single afferent (R_A) and efferent (R_E) arterioles. Colloid osmotic pressures (π) of plasma entering (π_A) and leaving (π_E) glomerular capillaries were estimated from C_A and C_E by using the equations derived by Deen et al [17]. In rats of Groups 2A and 2B only SNGFR, P_{GC} and P_T (Bowman's space hydraulic pressure) were measured.

In addition to these micropuncture measurements, timed urine samples were taken for measurement of whole kidney GFR. Also, blood samples were taken from the femoral arterial catheter at the beginning and at the end of each study period. These measurements were completed within approximately 20 minutes.

[1] The computation of various indices for glomerular microcirculatory dynamics is performed based upon the micropuncture measures obtained from different nephrons. The existence of heterogeneity of function among nephrons in remnant kidneys four weeks after subtotal nephrectomy is likely to cause an incorrect estimation of the various parameters, which can be obtained only by this computation. Therefore, in Groups 2A and 2B animals only SNGFR, P_{GC} and P_T, which can be assessed directly by micropuncture, were ascertained.

Table 2. Body weight, blood pressure, total kidney GFR and hematocrit of rats (Groups 1 and 2) in the present study

Group	Body wt *g*	MAP *mm Hg*	GFR *ml/min*	Hct *vol %*
1A (NPX + heparin)	141 ± 6[a]	147 ± 10[a]	0.36 ± 0.03[a]	39.7 ± 1.6[a]
1B (NPX + vehicle)	146 ± 4[a]	155 ± 6[a]	0.33 ± 0.04[a]	41.1 ± 1.1[a]
1C (Normal control)	173 ± 2	110 ± 1	0.86 ± 0.04[b]	44.9 ± 0.5
2A (NPX + heparin)	182 ± 10	139 ± 3[a,b]	0.49 ± 0.11[a,b]	42.9 ± 1.5
2B (NPX + vehicle)	171 ± 8	166 ± 8[a]	0.29 ± 0.05[a]	39.0 ± 1.4[a]

Values are expressed as mean ± SEM. Abbreviations are: body wt., body weight; MAP, mean systemic arterial pressure; GFR, whole kidney glomerular filtration rate; Hct, arterial hematocrit.

Values show a statistically significant ($P < 0.05$) difference from those of Group 1C ([a]); and between 1A vs. 1B, or 2A vs. 2B ([b]).

Analytical

Plasma and urine concentrations of inulin were determined by the anthrone method [18]. Details of the analytical procedures for inulin determination in nanoliter samples, and C_A and C_E measurements are given elsewhere [19, 20]. The volume of fluid collected from individual proximal tubules by micropuncture was estimated from the length of the fluid column in a constant-bore capillary tube of known internal diameter.

Calculations of glomerular hemodynamic parameters

Based upon the data obtained from the micropuncture study, SNGFR, SNFF, π_A, π_E, Q_A, Q_E, R_A, R_E, mean glomerular transcapillary hydraulic pressure differences (ΔP) and K_f were calculated using equations previously published [17, 21].

Activated partial thromboplastin time (aPTT)

To assess aPTT in rats with subtotal nephrectomy receiving heparin or vehicle, rat blood was obtained from the aorta two hours after the last dose of heparin (or vehicle) and immediately placed in 5 ml Vacutainer tubes (6418; Bectin-Dickinson & Co., Sunyvale, California, USA) containing sodium citrate. Cells were removed by centrifugation and the plasma was stored at −20°C before use. Plasma (100 μl) was incubated for three minutes at 37°C with 100 μl of prewarmed aPTT reagent (Thrombosil I; Ortho Diagnostic Systems Inc., Raritan, New Jersey, USA) in a fibrometer (Bectin-Dickinson & Co.). The clotting time (that is, the aPTT) was determined after addition of 100 μl of prewarmed 20 mM $CaCl_2$. While values for aPTT from six vehicle-treated rats averaged 30.3 ± 2.4 (1 SE) seconds, those from six heparin-treated rats were uniformly longer than 120 seconds.

Histological study

After exsanguination, tissue was obtained from the viable portion of the remaining kidney for weight determination and examination by light microscopy. Light microscopy was performed on periodic-acid-Schiff (PAS) stained sections obtained from the midline sagittal plane of the remnant kidney of each rat. The degree of abnormal glomerular histology was assessed in two ways. The first method utilized a variety of histological changes. Fifty consecutive glomeruli were examined at ×100 magnification in each rat, and the numbers of glomeruli with the following abnormal findings were counted: Grade 1 representing a glomerulus with PAS-positive mesangial granules and/or thickened mesangium; Grade 2, focal sclerosis with narrowed capillary loops; Grade 3, diffuse glomerular involvement with some capillary loops narrowed or obliterated. However, other capillary loops were opened with a thickened mesangium which contained PAS-positive granular substances; Grade 4, most or all loops obliterated and replaced by hyalinoid material. The second method evaluated the increase in mesangial matrix [22]. Fifty glomeruli from each kidney were graded from 1 to 4, with grade 1 representing PAS-positive mesangial lesion of ~25% of the glomerulus, and grade 4 representing involvement of 75 to 100% of the glomerulus. The average of the scores from all 50 glomeruli of a given kidney was calculated to obtain the sclerosis index for the single kidney.

Statistics

Statistical analysis was performed by analysis of variance (Tables 2 through 4) and the Bonferroni *t*-test (Table 5) for significance between groups, as appropriate. The null hypothesis was rejected when the *P* value was less than 0.05 [23].

Results

Systemic and whole kidney parameters in subtotally nephrectomized rats

Data for body weight, mean arterial blood pressure and total GFR obtained in rats of Groups 1A, 1B, 1C, 2A and 2B are shown in Table 2. When compared to Group 1C normal control rats, subtotally nephrectomized rats, treated or not, had mild to moderate systemic hypertension, and decreased whole kidney GFR two or four weeks after ablation of renal mass. Thus, the average values of MAP in rats of Groups 1A and 1B were 147 ± 10 and 155 ± 6 mm Hg as compared to 110 ± 1 mm Hg in Group 1C. GFR was markedly depressed in Group 1A (0.36 ± 0.03 ml/min) and Group 1B (0.33 ± 0.04 ml/min). Similar patterns were seen in rats of Groups 2A and 2B, which were studied four weeks after subtotal nephrectomy; however, levels of systemic blood pressure were higher in Group 2B rats than in Group 2A rats (Table 2). Total GFR was greater in Group 2A rats (0.49 ± 0.11 ml/min) than in rats of Group 2B (0.29 ± 0.05 ml/min).

Glomerular microcirculatory parameters two weeks after subtotal nephrectomy

Average values for several pertinent glomerular microcirculatory measurements in rats of Groups 1A, 1B and 1C are summarized in Table 3. The remnant nephrons of Group 1A heparin-treated and 1B non-treated rats were characterized by marked glomerular hyperfiltration and hyperperfusion; mean values for SNGFR and Q_A in Group 1A (58.9 ± 6.4 and 229 ± 41 nl/min, respectively) and Group 1B (60.8 ± 7.8 and 176 ± 13

Table 3. Summary of various glomerular microcirculatory parameters in normal rats and rats 2 weeks after subtotal nephrectomy

Group	SNGFR	P_{GC}	ΔP	P_E	C_A	Q_A	R_A	R_E	K_f	SNFF
	nl/min	*mm Hg*			*g/dl*	*nl/min*	$\times 10^{10}$ *dyn · cm^{-5} nl/(sec · mm Hg)*			
1A (NPX + heparin)	58.9 ± 6.4[a]	71 ± 4[a]	51 ± 2[a]	25 ± 2[a]	5.4 ± 0.1[a]	229 ± 41[a]	1.68 ± 0.37[a]	1.34 ± 0.27[a]	0.094 ± 0.083[a]	0.28 ± 0.03
1B (NPX + vehicle)	60.8 ± 7.8[a]	73 ± 4[a]	54 ± 3[a]	23 ± 2[a]	5.4 ± 0.1[a]	176 ± 13[a]	2.22 ± 0.21[a]	1.72 ± 0.15	0.033 ± 0.008	0.34 ± 0.03
1C (Normal control)	20.8 ± 1.9	46 ± 1	32 ± 1	18 ± 1	6.2 ± 0.2	66 ± 12	5.08 ± 0.90	1.90 ± 0.37	0.049 ± 0.008	0.35 ± 0.04

Values are expressed as mean ± SEM. Abbreviations are: SNGFR, single nephron glomerular filtration rate; P_{GC}, glomerular capillary hydraulic pressure; ΔP, glomerular transcapillary hydraulic pressure difference; P_E, surface efferent arteriolar hydraulic pressure; C_A, afferent arteriolar plasma protein concentration; Q_A, single nephron plasma flow rate; R_A, afferent arteriolar resistance; R_E, efferent arteriolar resistance; K_f, ultrafiltration coefficient; SNFF, single nephron filtration fraction.

[a] $P < 0.05$ vs. Group 1C. No statistically significant ($P > 0.05$) difference between Group 1A vs. Group 1B was detected in any of the parameters shown.

Table 4. Various glomerular microcirculatory parameters in normal rats and rats 4 weeks after subtotal nephrectomy

Group	SNGFR	P_{GC}	ΔP	P_T
	nl/min	*mm Hg*		
2A (NPX + heparin)	83.3 ± 7.1[a]	65 ± 2[a]	48 ± 2[a]	18 ± 2
2B (NPX + vehicle)	63.7 ± 6.5[a]	62 ± 4[a]	47 ± 4[a]	16 ± 1
1C (Normal control)	20.8 ± 1.9	46 ± 1	32 ± 1	14 ± 1

Values are expressed as mean ± SEM. Abbreviations are: SNGFR, single nephron glomerular filtration rats; P_{GC}, glomerular capillary hydraulic pressure; ΔP, glomerular transcapillary hydraulic pressure difference; P_T, Bowman's space hydraulic pressure.

[a] $P < 0.05$ vs. Group 1C. No statistically significant ($P > 0.05$) difference between Group 2A vs. Group 2B was detected in any of the parameters shown.

Table 5A. Semiquantitative analysis of glomerular structural changes in rats 7.5 weeks after subtotal nephrectomy with a use of a grading scale which incorporates various forms of abnormalities

	Normal	Grade 1	Grade 2	Grade 3	Grade 4
3A (heparin)	93 ± 2	3 ± 1	2 ± 1	2 ± 1	0.5 ± 0.3
3B (untreated)	23 ± 7	34 ± 6	4 ± 1	27 ± 6	12 ± 4
P value <	0.0001	0.0001	0.10	0.001	0.02

Grades 1–4 are defined in the **Methods** section. Values are expressed as a percentage of glomeruli examined (mean ± SEM).

Table 5B. Semiquantitative expression of glomerular structural changes in rats 7.5 weeks after subtotal nephrectomy with a use of a grading scale which focuses on the mesangial matrix

Group	Glomerular sclerosis index
3A (heparin)	0.23 ± 0.04
3B (untreated)	0.40 ± 0.05
P value <	0.01

Fifty glomeruli from each kidney were evaluated by grading them from 0 to 4, with 1 representing PAS-positive mesangial lesion of ~25% of the glomerulus, and 4 representing lesion of 75 to 100% of the glomerulus. The average of the scores from all 50 glomeruli of a given kidney was calculated to obtain the sclerosis index for the single kidney.

nl/min, respectively) were substantially higher than those measured in Group 1C normal rats (20.8 ± 1.9 and 66 ± 12 nl/min). Also, the remnant nephrons of both Groups 1A and 1B rats had markedly and comparably elevated P_{GC}, averaging 71 ± 4 and 73 ± 4 mm Hg, respectively. Likewise, values of ΔP in Groups 1A (51 ± 2 mm Hg) and 1B (54 ± 3 mm Hg) were similar and considerably elevated over normal control levels (32 ± 1 mm Hg). In contrast to these abnormally high levels of pressure, Group 1A animals (heparin treated) were characterized by having substantially increased K_f values, averaging 0.094 ± 0.083 nl/(sec · mm Hg), compared with 0.049 ± 0.008 nl/(sec · mm Hg) measured in Group 1C normal rats. On the other hand, the mean K_f value of Group 1B vehicle-treated rats tended to be lower than that of Group 1C normal rats. It is clear from Table 3 that the high P_{GC} values in both Groups 1A and 1B resulted largely from a reduction in R_A. The observed patterns of glomerular hypertension, hyperperfusion and hyperfiltration in remnant nephrons of Group 1B rats essentially duplicate those reported by us [11] and others [3, 8] previously. Moreover, essentially identical glomerular hemodynamic patterns were observed in Group 1A (heparin-treated rats) as in Group 1B when assessment was made two weeks after subtotal nephrectomy.

Glomerular microcirculatory parameters four weeks after subtotal nephrectomy

Average values for SNGFR, P_{GC}, ΔP and P_T measured in rats of Groups 2A and 2B are summarized in Table 4. The remnant nephrons of Group 2A, heparin-treated and 2B, vehicle-treated rats, four weeks after subtotal nephrectomy were also characterized by marked glomerular hyperfiltration and hypertension. Values for SNGFR and P_{GC} in Group 2A (83.3 ± 7.1 nl/min and 65 ± 2 mm Hg, respectively) and Group 2B (63.7 ± 6.5 nl/min and 62 ± 4 mm Hg, respectively) were substantially higher than those measured in normal rats; however, the P_{GC} values measured at four weeks were somewhat lower than those measured at two weeks (Table 3). Due to the abnormally high P_{GC}, the values for ΔP in Groups 2A (48 ± 2 mm Hg) and 2B (47 ± 4 mm Hg) were similar and substantially elevated over normal control

levels, although Group 2A and 2B rats had modestly elevated P_T, with values averaging 18 ± 2 and 16 ± 1 mm Hg, respectively. Collectively, again, there was no effect of heparin treatment on any of the glomerular hemodynamic parameters measured in remnant nephrons four weeks after subtotal nephrectomy.

Glomerular light microscopic findings in remnant nephrons 7.5 weeks after subtotal nephrectomy

The glomerular histological alterations discernible on light microscopy in rats with subtotal nephrectomy have been described [2]. The glomerular involvement is focal and consists of segmental thickening of capillaries, cellular proliferation, accumulation of proteinaceous material, occlusion of capillaries and structureless glomeruli [2, 7]. Focal interstitial inflammatory cell infiltration and dilated tubules containing hyaline casts are usually present. The glomerular structure of Group 3B untreated rats 7.5 weeks after subtotal nephrectomy possesses all of the above features typical for remnant nephrons. Thus, as shown in Table 5A, whereas Group 3B rats had only 23 ± 7% normal glomeruli, Group 3A heparin-treated rats had an average value of 93% normal glomeruli. Moreover, in Group 3B untreated rats 12 ± 4% of the glomeruli were Grade 4, those with the most severe glomerular abnormalities, contrasting to Group 3A's only 0.5 ± 0.3%. Occasionally, periglomerular fibrosis and "onion ring" lesions were seen in Group 3B rats, while such severe changes were not present in any of the specimens from Group 3A animals.

Table 5B summarizes the results from another semiquantitative histological analysis, which focused only on the glomerular mesangial lesion. On average, therefore, the heparin treatment attenuated the expansion of mesangial region by roughly a half in the remnant nephrons. Despite the fact that these two analyses given in Tables 5A and 5B were conducted by different investigators using two different semiquantitative scales, a uniform notion emerges, namely heparin exerted a significant protective action on the glomerular histology of the remnant nephrons.

Discussion

Based on the marked functional and structural internephron heterogeneity seen in many renal diseases of humans and animals [12, 13, 24–26], it has been speculated that the progressive destruction of the renal architecture, which proceeds often relentlessly in these conditions, stems from the loss of a crucial number of functioning nephrons during the initial pathologic insult. The reduction in nephron population may lead to functional adaptations, such as an increase in glomerular filtration, in the remaining "intact" nephrons. If such functional adaptations are harmful and responsible for further deterioration in the structure and function of these initially "healthy" nephrons, then it is conceivable that progressive destruction of the whole kidney can occur even after the initial pathologic insult is eliminated [1, 3, 5]. Subtotal nephrectomy (or remnant kidney model), which simulates experimentally this hypothetical initial reduction in nephron population, leads to structural changes of remnant glomeruli in a manner reminiscent histologically of the end-stage kidneys seen in man with progressive renal disase [1–3]. Alterations in the function of the remnant nephrons are also noted soon after nephrectomy, including proteinuria and a compensatory increase in glomerular filtration rate. Recent studies have further documented an elevation in glomerular pressure and plasma flow rate in the remnant nephrons [3]. Certain experimental maneuvers, such as dietary manipulation of protein intake, were found to modify both the magnitude of the early changes in glomerular hemodynamics and the later structural abnormalities [3, 8, 9, 27]. This observation led the investigators to postulate that increased glomerular perfusion, filtration and/or elevation of glomerular capillary pressure may act as the initial step in the extensive damage of glomeruli seen in many chronic diseases of the kidney.

Intraglomerular coagulation is potentially another factor which may play a role in the progressive destruction of the glomerular architecture in chronic renal diseases [28]. Aggregation of platelets as a result of glomerular capillary endothelial injury or in response to substances locally released from invading or resident cells, may cause release of platelet products, such as platelet-derived growth factor which, in turn, may stimulate proliferation of mesangial cells and increase the production of mesangial matrix. Platelet aggregation may also cause intraglomerular coagulation. Previous studies by us and others, using the model of subtotal nephrectomy, have demonstrated that heparin administration to these animals markedly attenuates the development of glomerular structural damage [2, 6, 14]. Heparin exerts a variety of biological actions through its potent inhibitory effect on several serine proteases, including clotting factors IX, X, XI, XII, thrombin [29, 30], as well as kallikrein, a substance known to act as a vasodilator [31, 32]. When rats with subtotal nephrectomy were given heparin, the development of systemic hypertension routinely seen in these animals was markedly attenuated [2, 6, 14]. This raised the possibility that heparin ameliorated the development of glomerulosclerosis in the remnant kidney by its ability to prevent the changes in glomerular hemodynamics (that is, increased flow and intraglomerular pressure) which occur in the remnant nephrons.

In the present study heparin, administered to subtotally nephrectomized rats in a fashion identical to that employed in our previous studies [2, 6], had a remarkable protective effect on glomerular structure. As discussed in the **Results** section, the micropuncture measurements obtained in the current study indicate that this protective effect of heparin cannot be attributed to a potential effect of the drug in modifying the abnormal glomerular hemodynamics, since heparin treatment had no discernible influence on intraglomerular pressures and flows. At two weeks, the magnitude of the increase in glomerular pressure, glomerular perfusion and filtration was comparable between the two groups, that is, P_{GC}, Q_A and SNGFR were equally elevated above normal control levels in both heparin-treated (Group 1A) and vehicle-treated (Group 1B) animals (Table 3). Although only glomerular pressure and SNGFR were assessed, similar changes were also present at four weeks. Thus, SNGFR and P_{GC} of remnant nephrons were again markedly elevated in heparin-treated and vehicle-treated rats (Table 4). In contrast to this lack of a heparin effect on P_{GC}, the severe systemic arterial hypertension which developed in untreated rats was remarkably attenuated in heparin-treated rats (Table 2). It appears, therefore, that the high pressure prevailing at the level of glomerular capillaries in remnant nephrons is the

consequence primarily of local adjustments of vasomotor tone occurring within the glomerular microcirculatory system rather than being a direct reflection of the changes in systemic blood pressure.

It is beyond the scope of our results to speculate whether the abnormal glomerular hemodynamics, which occur in this animal model, indeed mediate the structural injury of the glomerulus. Instead, our observations demonstrate a hemodynamic-independent, and remarkably efficient protective effect of heparin on the glomerular structure. This indicates an involvement in the pathophysiological process of glomerular damage of one or more steps which are highly sensitive to the biological actions of heparin. Inhibition of these "heparin-sensitive" processes can preserve the glomerulus virtually free of structural abnormalities, even in the presence of the potentially injurious effect of increased intraglomerular pressures and flows. The role of an anticoagulant effect of heparin in the preservation of glomerular structure of remnant nephrons was suggested by a recent study of Olsen [14]. In her study, a low molecular weight fraction of heparin, which had lost a substantial degree of anticoagulant activity, exerted virtually no protective effect on remnant nephrons, contrasting with the dramatic glomerular structure-sparing effect observed with the administration of standard heparin. It is possible, however, that besides anticoagulation, other biological properties of heparin were lost in the preparation of the low molecular weight fragment of heparin. We have found that N-desulfated heparin devoid of anticoagulant activity also protects the glomerular structure in rats with a remnant kidney [28]. Consequently, other biological effects of heparin besides anticoagulation may play a role in its protective effect on glomerular structure.

The anti-proliferative properties of heparin have recently been the focus of great interest on the part of several investigators. In a study using cultured rat mesangial cells, Castellot et al [33] showed that heparin added to the culture media suppressed the growth of mesangial cells in a dose-dependent manner. Moreover, the culture media of glomerular epithelial cells was shown in their study to contain inhibitor(s) of mesangial cell growth, which could be destroyed by heparinase. Endothelial cells also appear to produce similar heparin-like substances. More recently, in an in vivo study, Coffey and Karnovsky [34] reported that a non-anticoagulant heparin derivative prepared by ion exchange chromatography failed to inhibit the mesangial cell proliferation which was induced experimentally by administration of *habu* snake venom, whereas anticoagulant heparin treatment markedly attenuated mesangial cell proliferation. Although the mechanism involved in the anti-proliferative (or anti-inflammatory) action of heparin is currently unknown, it is tempting to speculate that this unique property of heparin may underlie its potent structure-sparing effect on the glomerulus of remnant nephrons in view of the notion that expansion of the mesangial area within the glomerular architecture is a prerequisite for the subsequent development of glomerular sclerosis.

Although commonly considered to be less specific, the anionic properties of heparin, which is a highly negatively-charged molecule, may also play a role in protecting the glomerular structure of remnant nephrons. While the loss of anionic charge and the distortion of normal configuration of glomerular epithelial cells or glomerular basement membrane precede the structural abnormalties of other glomerular components, including mesangial cells, in various forms of glomerular sclerosis in human and experimental animals [35–39], treatment with heparin has often been shown to ameliorate such changes on epithelial cells and other glomerular structural components [40]. Thus, it is conceivable that heparin may exert its effect on remnant glomeruli by interfering with the loss of negative charge and distortion of epithelial cells occurring in the early phases of subtotal renal ablation. In this regard, given the fact that the anionic sites of both epithelial cells and glomerular basement membrane consist primarily of heparan sulfate, a class of glycosaminoglycan to which heparin belongs, heparin administration may potentially be replacing a decrease in endogenous heparan sulfate and be a highly efficient therapeutic maneuver through this mechanism.

In summary, through one or more of the mechanisms discussed above, heparin effectively prevents glomerular structural damage in remnant nephrons in a model which is otherwise characterized by severe and relentlessly progressive destruction of glomeruli. The present study demonstrates that this protective effect of heparin is channelled through hemodynamic-independent mechanisms. Finally, our observation that attenuation of glomerular structural damage in remnant nephrons by heparin treatment is accompanied by amelioration of severe systemic hypertension without a decrease in intraglomerular pressure, which otherwise characterizes the glomerular hemodynamic changes in this animal model, indicates that systemic hypertension is a consequence of the renal dysfunction rather than, as suggested by others [14], a cause of the destruction of glomerular structure.

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Appendix. Abbreviations

C	Protein concentration, g/dl
GFR	Glomerular filtration rate (whole kidney inulin clearance), ml/min
K_f	Glomerular capillary ultrafiltration coefficient, nl/(sec · mm Hg)
MAP	Mean arterial pressure, mm Hg
NPX	Nephrectomy
P	Mean pressure, mm Hg
ΔP	Mean transcapillary hydraulic pressure difference, mm Hg
π	Colloid osmotic pressure, mm Hg
Q_A	Initial glomerular plasma flow rate, nl/min
R	Resistance to blood flow, $\times 10^{-10}$ dynes · sec · cm^{-5}

SNFF Single nephron filtration fraction
SNGFR Single nephron glomerular filtration rate, nl/min

Subscripts

A Femoral artery or afferent arteriole
E Efferent arteriole
GC Glomerular capillary
T Proximal tubule or Bowman's space

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